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DETERMINATION OF 3,6-DICHLOROPICOLINIC ACID RESIDUES IN SUGAR BEETS BY GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

A method is described for the determination of 3,6-dichloropicolinic acid in sugar beets using a gas chromatographic method with either a capillary column and electron capture detection or a packed column and a Hall electrolytic conductivity detector. 3,6-Dichloropicolinic acid was extracted from sugar beets by potassium hydroxide and after partitioning in ether, derivatized as its methyl ester by diazomethane. The sensitivity of the method is *ca.* 0.05 mg/kg of 3,6-dichloropicolinic acid.

INTRODUCTION

3,6-Dichloropicolinic acid is a foliar-applied growth-regulator herbicide, manufactured by the Dow Chemical Company under the trade mark "Lontrel"[1]. It is readily absorbed by leaves and roots and translocated throughout the plant. It is used for weed control in cereals and sugar beets[2]. 3,6-Dichloropicolinic acid is chemically related to an other herbicide, picloram, but is less persistant in soil.

The methods available for determining picloram have led to the development of various methods for 3,6-dichloropicolinic acid, particularly in soils[3–6], all of which are based on a derivatization of 3,6-dichloropicolinic acid as its methyl ester by diazomethane, or as its butyl ester by butanol and sulphuric acid esterification. However, reproducibility and accuracy of these methods are not always satisfactory.

The method reported here is based on capillary-column gas–liquid chromatography (GLC) with electron-capture detection (ECD). Some tests have been also done using packed-column GLC with a Hall electrolytic conductivity detector in the chlorine mode[7–9].

EXPERIMENTAL

Reagents

3,6-Dichloropicolinic acid was kindly supplied by Dow Chemical (Midland, MI, U.S.A.). All solvents and reagents were of chromatographic grade or for residue analysis and were checked before use.

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Preparation of diazomethane

In order to avoid distillation of diazomethane, which is a dangerous procedure, the present method involves the generation of diazomethane, by the action of alkali on N-methyl-N-nitroso-N′-nitroguanidine. The gas is collected in diethyl ether. The whole apparatus is sealed during the preparation. In detail, 120 mg of N-methyl-N-nitroso-N′-nitroguanidine (Fluka 68051) are placed in the bottom of the inside tube of a Chrompack diazomethane generator (millimole size, cat. No. 12421). Through the screw-cap opening, 500 µl of water are added. All the reagent must be in the bottom of the tube and not on the wall, to ensure complete reaction. Then 3 ml of ether are poured in the outside tube and the two parts of the apparatus are assembled and held firmly with a pinchtype clamp. The lower part is immersed in an ice bath, and 600 µl of 5 *M* sodium hydroxide are injected through the teflon rubber septum with a narrow-gauge needle syringe. The reaction is carried out for 45 min. The reagent must be freshly prepared and kept until use in a freezer.

Always use an efficient fume hood and appropriate safety precautions when handling diazomethane.

Standard pesticide solutions

Stock solution is prepared by dissolving 0.1000 g of pure 3,6-dichloropicolinic acid in ether in a 250-ml volumetric flask and making up to volume with ether. It is stored in a freezer. From this stock solution, dilute standards (0.1–10 mg/l) in ether are prepared as required.

Apparatus

Capillary-column GLC. The GLC apparatus is a Hewlett-Packard 5880 A, level four, equipped with a ^{63}Ni ECD and a split-splitless injector. A wide-bore flexible capillary column, 25 m × 0.20–0.21 mm I.D., coated with methyl silicone fluid (Carbowax 20M deactivated, Hewlett-Packard 19091-60010) is used

Packed-column GLC. The apparatus is a Tracor 560 equipped with the Hall electrolytic conductivity detector, Tracor 700, in the chlorine mode. A vent valve protects the detector furnace tube from contamination by repetitive injections of solvent. The detector signal is measured in terms of peak area by a Hewlett-Packard integrator automation system 3385 A. A silanized glass column (90 cm × 2 mm I.D.) is packed with 5% OV-210 and 2% OV-17 on Gas-Chrom Q, 80–100 mesh.

Chromatographic conditions

Capillary-column GLC. Suitable resolution of 3,6-dichloropicolinic acid methyl ester is obtained under the following conditions: injector temperature, 250°C; detector temperature, 250°C; oven temperature programme: 60°C for 0.50 min, then at 30°C/min final value of 130°C, held for 10.00 min; column clean-up, 220°C for 13 min; injection volume 1 µl by splitless (0.50 min after injection without splitting).

The carrier gas is high purity helium at an inlet pressure of 10 p.s.i. and a flow-rate through the column of 2 ml/min.

The auxiliary detector gas is argon–methane (95:5) at an inlet pressure of 30 p.s.i. and a flow-rate of 30 ml/min.

Packed-column GLC. Injector temperature, 200°C; oven temperature, 165°C; transfer-line temperature, 200°C; carrier gas, high purity helium with a flow-rate of 40 ml/min.

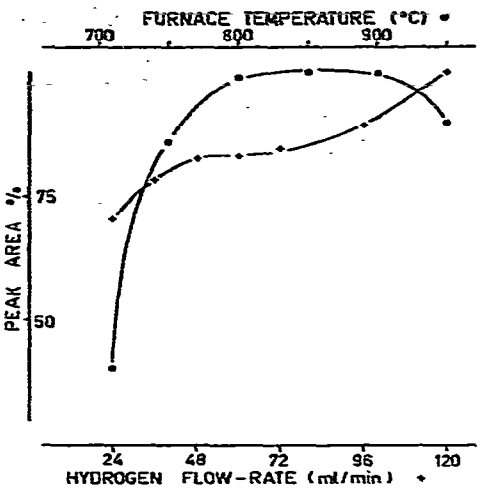

Fig. 1. Hall electrolytic conductivity detector furnace temperature (●) and flow-rate (+) profiles of 3,6-dichloropicolinic methyl ester acid.

The catalytic reduction is conducted in an empty quartz combustion tube (19 cm × 0.6 cm O.D.) heated in a furnace at 850°C and swept by hydrogen with a flow-rate of 60 ml/min.

Fig. 1 shows the furnace temperature (hydrogen flow-rate 60 ml/min) and hydrogen flow-rate (furnace at 850°C) profiles for dichloropicolinic acid derivative. The hydrogen flow-rate is fixed at 60 ml/min to ensure an acceptable background level and good reproducibility.

The vent valve is turned off 1 min after the injection. The electrolytic solution contains 40% 1-propanol in distilled water; its flow-rate is 0.8 ml/min, and 8 μl are injected.

Methods

Sugar beets extraction. Sugar beets (roots, tops or leaves) are cut with a food-cutter "Hobart", and 50 g of the homogeneous sample are blended for 5 min at high speed with 150 ml of 0.25 *M* potassium hydroxide in a "Sorvall Omni-Mixer". The mixture is transferred to a 500-ml centrifuge bottle, rinsed with 0.25 *M* potassium hydroxide and centrifuged for 5 min at 4000 *g*. The supernatant is poured into a 750-ml separatory funnel after filtration through a Schleicher and Schüll 597 filter paper. The bottom of the centrifuge tube is extracted again with 100 ml of 0.25 *M* potassium hydroxide, and the extract is centrifuged and filtered. The supernatants are combined in the same separatory funnel. Then 100 ml of ether, 30 g of sodium chloride and 70 ml of 4 *M* sulphuric acid are added to the funnel. After vigorous shaking and separation of the layers, the aqueous layer is poured into another separatory funnel and the ether layer is transferred to a 250-ml centrifuge bottle. After centrifugation, the ether is dried through a 4-cm bed of anhydrous sodium sulphate and collected in a 300-ml erlenmeyer flask. The aqueous layer is extracted again with 50 ml of ether. The ether layer is transferred to the same centrifuge bottle which, after vigorous shaking, is

centrifuged. After drying on the anhydrous sodium sulphate bed, it is added to the same 300-ml erlenmeyer flask.

Derivatization. The ether extract is evaporated to 5–10 ml in a vacuum rotary evaporator at 30°C, quantitatively transferred to a 25-ml erlenmeyer flask with ether and evaporated to dryness under a dry air stream. Then 7 ml of the diazomethane solution are added and, after swirling, the erlenmeyer flask is kept at 4°C for 2 h.

The diazomethane is evaporated under a gentle stream of dry air without any heating. The dry residue is kept in a freezer until use.

At the time of injection, the residue is dissolved in 10 ml of hexane–ether (70:30) and injected into the gas chromatograph.

RESULTS AND DISCUSSION

Stability of 3,6-dichloropicolinic acid and its methyl ester

The stability of 3,6-dichloropicolinic acid was tested at 4°C in hexane, ether and dry sugar beets extracts, and in deep-frozen leaves and roots. No degradation was observed after one month.

The methyl ester was stable for one month at 4°C in hexane, ether and dry sugar beets extracts, but volatilization was observed at higher temperatures. Above 30°C, this volatilization is very important (losses greater than 50% after 10 min). This must be take into account, particularly during the evaporation or concentration steps.

Extraction

In comparison with the previous methods, one of the main modifications in the present one is the use of all the analytical samples without any dilution: 50 g of sugar beets is the analytical sample and an extract of the entire sample 50 g is injected into the gas chromatograph. This ensures a high sensitivity of the method and avoids some analytical errors, particularly in routine analysis.

Centrifugation of the blended sample allows good recovery of potassium hydroxide and facilitates the second extraction. Table I shows the advantages of this second extraction. A third extraction doesn't improve the results. This centrifugation procedure can be replaced, in routine analysis, by filtration of the blended mixture under suction on a 1-cm Celite 545 layer, followed by two washes with 50 ml of 0.25 *M* potassium hydroxide and two washes with 50 ml of water to destroy the foam,

TABLE I

RECOVERY OF THE EXTRACTION PROCEDURE BY CENTRIFUGATION

Spiked sample at mg/kg	*Recovery (%) after*		
	*One extraction**	*Two extractions**	*Three extractions**
1.10	85.2	94.1	94.6
0.55	87.1	93.4	93.3
0.05	86.8	92.1	92.5

* With 100 ml of potassium hydroxide solution.

TABLE II

RECOVERY OF THE EXTRACTION PROCEDURE BY FILTRATION

Spiked sample at mg/kg	*Recovery (%) after*			
	Two KOH washes	*Three KOH washes*	*Two KOH washes and two H_2O washes*	*Two KOH washes and three H_2O washes*
1.10	88.4	88.2	90.0	90.2
0.55	87.1	87.4	89.5	90.1
0.05	85.7	85.0	89.5	89.4

especially with leaf extracts. However, this procedure gives lower recovery, as shown in Table II.

The acidity during the ether extraction is another important parameter. A good yield from this extraction is obtained with 4 *M* sulphuric acid, as shown in Table III. Sometimes, a lower acidity gives lower recovery and poor reproducibility. After this extraction, it is essential to centrifuge the ether layer to destroy the strong emulsion and obtain a clear extract.

Derivatization

Fig. 2 shows the amount of diazomethane required for a correct derivatization of pure 3,6-dichloropicolinic acid and of herbicide in sample extracts. If 500 μl of diazomethane are enough to derivatize 500 μg of acid, then 7 ml are required for a sample extract. Usually 5 ml of diazomethane would be enough, but in some cases, *e.g.* with a high percentage of sugar, 7 ml are necessary. Use of more diazomethane should be avoided because it is expensive and it has sometimes been observed to degrade methyl esters.

The time of contact between diazomethane and 3,6-dichloropicolinic acid is another critical point, as shown in Fig. 3. The right length of time to ensure complete derivatization without degradation seems to be 2 h.

TABLE III

INFLUENCE OF SULPHURIC ACID MOLARITY ON RECOVERY AND REPRODUCIBILITY OF ETHER EXTRACTION OF SUGAR BEETS EXTRACTS SPIKED AT 1 mg/kg OF 3,6-DICHLOROPICOLINIC ACID

Sample	*Recovery (%)*			
	Sulphuric acid molarity			
	2 M	*3 M*	*4 M*	*5 M*
1	67.5	90.5	93.2	94.5
2	80.2	93.2	94.0	92.1
3	85.4	87.1	92.7	93.7
4	84.1	92.1	94.8	94.0
5	77.8	93.5	93.5	—

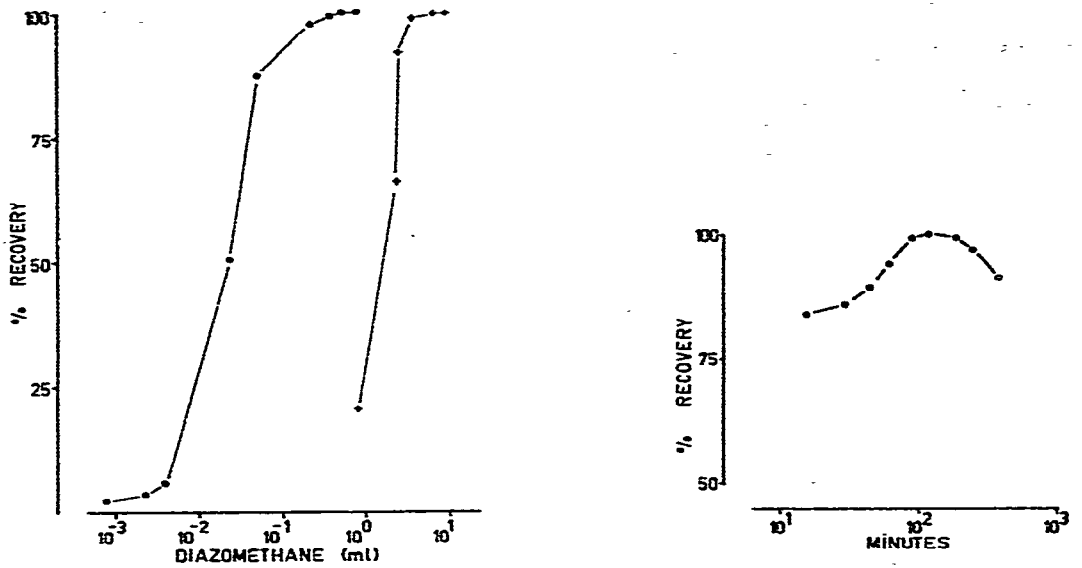

Fig. 2. Amounts of diazomethane required for derivatization of 3,6-dichloropicolinic acid pure (●) and in sugar beets extract (+).

Fig. 3. 3,6-Dichloropicolinic methyl ester synthesis: influence on recovery of the time contact of diazomethane with acid.

Gas chromatography

Typical packed-column and capillary-column chromatograms of crop extract containing 3,6-dichloropicolinic acid are shown in Fig. 4.

The advantage of the Hall electrolytic conductivity detector is its selectivity for chlorine compounds. This selectivity reduces considerably the interfering peaks and the analysis time (less than 4 min). However, for acceptable results, the detector must be very carefully conditionned, which is difficult to achieve with the Hall detector Tracor 700. The new micro Hall Tracor is probably better for trace analysis. Packed-column GLC does not ensure a perfect separation of all impurities of sample extract.

The capillary column with ECD is very satisfactory. The column ensures a very good separation of interfering peaks from picolinic methyl ester acid, and the reproducibility is excellent. Standard deviations for three injections of the same sample extract spiked at 1.10, 0.55 and 0.055 mg/kg were 1.7, 2.2 and 4.9%, respectively. Under the conditions used, the ^{63}Ni electron capture detector gave a lower detection level in extracts: 250 pg as against 5 ng by the Hall electrolytic detector.

It is also possible to work with a 12-m fused silica capillary column coated with methyl silicone fluid, with an oven start temperature of 60°C for 0.5 min, followed by a programme rate of 30°C/min to 130°C for 4 min. A column clean-up at 220°C for 10 min is suitable. These parameters reduce the analysis time and give good results. However, the peak separation is poorer.

Recovery

The method described in this paper gives recoveries near 90%. Table IV shows

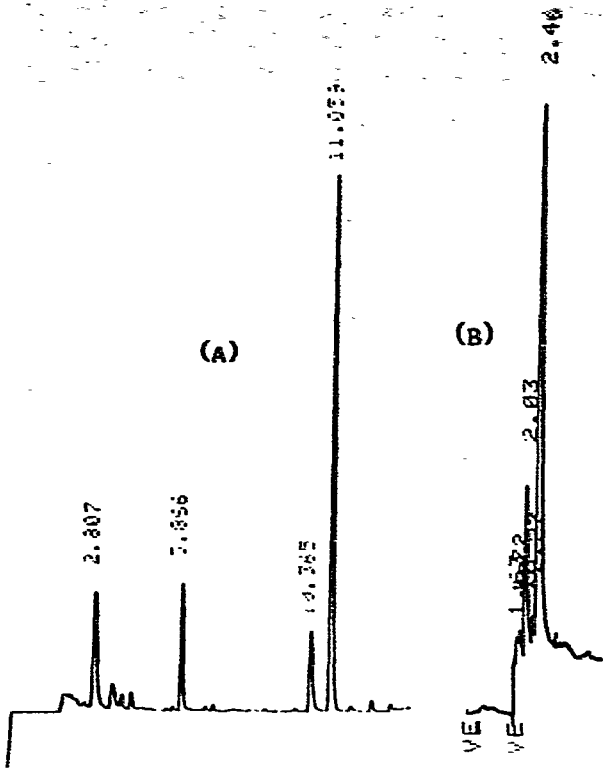

Fig. 4. Chromatograms from (A) a capillary column of 10 μg of dichloropicolinic acid in sugar beets extract, and (B) a packed column of 50 μg of dichloropicolinic acid in sugar beets extract. Detection: (A) ECD; (B) Hall electrolytic conductivity detector.

TABLE IV

DETERMINATION OF 3,6-DICHLOROPICOLINIC ACID IN SUGAR BEETS SPIKED AT VARIOUS LEVELS

Sample	*Recovery (%)*		
	Samples spiked at		
	1.10 mg/kg	*0.55 mg/kg*	*0.055 mg/kg*
1	98.3	95.9	98.2
	92.8	96.9	80.0
2	97.9	92.5	83.6
	93.4	85.4	94.5
3	90.1	90.2	85.4
	95.7	98.4	92.7
4	91.8	98.2	83.6
	94.1	90.5	96.4
Mean recovery	94.3	93.5	89.3
Variance	7.238	18.74	41.86
Standard deviation	2.8%	4.6%	7.2%

the recovery and reproducibility of sample extracts spiked at various levels. Standard deviations for spiked samples at 1.10–0.05 mg/kg range from 2.8 to 7.2%. The detection limit is 0.05 ppm (50 μg of 3,6-dichloropicolinic acid per kilogram of sugar beets).

CONCLUSIONS

The method described yields satisfactory results. The most critical step is the derivatization and evaporation, but when all the parameters are well known there are no difficulties. The selectivity of the Hall detector and the resolution of the capillary column ensure a perfect identification and quantitation of 3,6-dichloropicolinic acid residues without additional clean-up. It would be interesting to couple capillary GLC with the Hall electrolytic conductivity detector, but at present our laboratory is not equipped to do so.

The method can be also applied to the determination of residues in water, when it can be simplified by elimination of the centrifugation or filtration steps.

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